AD_____

Award Number: W81XWH-06-1-0768

TITLE: Complement and Immunotherapy of Breast Cancer

PRINCIPAL INVESTIGATOR: Michelle Rapisardo
Stephen Tomlinson, Ph.D.

CONTRACTING ORGANIZATION: Medical University of South Carolina
Charleston, SC 29403

REPORT DATE: October 2008

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| 1 October 2008 | Annual Summary | 15 Sep 2007 – 14 Sep 2008 |

**4. TITLE AND SUBTITLE**

Complement and Immunotherapy of Breast Cancer

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-06-1-0768

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Michelle Rapisardo
Stephen Tomlinson, Ph.D.

E-Mail: rapisar@musc.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Medical University of South Carolina
Charleston, SC 29403

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
In general, monoclonal antibody immunotherapy for cancer has fallen short of clinical expectations. This is due, at least in part, to the over expression of membrane bound complement inhibitors on the tumor cell surface. We proposed to prepare and investigate the effects of two novel recombinant proteins aimed at modulating complement to increase the immune response to breast tumors. During year two it was proposed to finish construction, expression and purification of CR2Fc, characterize the recombinant protein *in vitro* and begin *in vivo* studies. Construction and purification of CR2Fc has been accomplished. The purification of the protein was optimized and stocks of protein have been produced for these studies. The protein has been characterized *in vitro* and shown to bind C3 deposited on tumor cells. Technical difficulties were encountered *in vitro* when looking at increased C3 deposition and tumor cell lysis but are currently being resolved. We will finish *in vitro* studies and proceed with *in vivo* studies to determine the effect CR2Fc has on the immune response and whether it is protective against breast tumors.

**15. SUBJECT TERMS**
complement, immunotherapy, monoclonal antibody

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 7 | **19b. TELEPHONE NUMBER** *(include area code)* |

## Table of Contents

**Introduction**

In general, a naturally occurring or therapeutically administered humoral response to tumor is not effective. In particular, antibody immunotherapy of cancer has fallen short of clinical expectations. This is due, at least in part, to the upregulation of complement inhibitory proteins on the tumor cell surface. It is hypothesized that overcoming the complement resistance of breast tumor cells will increase the efficacy of antibody immunotherapy. To this end, we proposed to prepare and characterize two novel fusion proteins aimed at modulating complement to enhance the humoral immune response and potentially induce a cellular immune response against the breast cancer antigen MUC1.

The recombinant protein CR2Fc consists of complement receptor 2 (CR2) linked to the Fc region of mouse IgG2a. Following monoclonal antibody therapy complement is activated and deposited on tumor cells; the CR2 portion of the recombinant protein is expected to target to the site of complement activation and will therefore serve as the targeting domain. The Fc portion is expected to increase complement activation, therefore further generating CR2 ligands and increasing complement dependent effector mechanisms such as CDC and CDCC as well as Fc effector mechanisms such as ADCC.

**Expression and Purification of CR2Fc** (Task 2)

Due to a technical difficulty during year one, expression and purification of CR2Fc was not completed. However, during year two this was overcome and CR2Fc was successfully expressed and is continuously being purified. Two different CR2Fc constructs have been created, one with CR2 connected to the Fc portion without a linker (CR2Fc no linker) and one with CR2 connected to the Fc portion with a linker (CR2Fc linker). A Protein A column is being used for purification and the average yield is 13 mg/L of supernatant. The purity of the recombinant protein was verified by SDS-Page (data not shown) and the identity of the purified protein was confirmed by SDS- page and western blotting. The western blot was stained with 7G6 (an anti-mouse CR2 monoclonal antibody) and a band at the expected molecular weight of 54 Kd was seen (Figure 1).

***In vitro* characterization of CR2Fc** (Task 3)

CR2Fc has been characterized *in vitro* for binding to complement component C3 deposited on cells, its ability to increase C3 deposition on the cell surface and its ability to increase cell lysis. Using a flow cytometric binding assay, it was shown that both CR2Fc constructs (with and without linker) bind with high affinity and target to C3 deposited on CHO cells (Figure 2). In the absence of serum (and therefore C3) CR2Fc did not bind to the cells, indicating that it is targeting specifically to deposited C3 (data not shown). We then investigated the ability of CR2Fc to increase C3 deposition on the cell surface by flow cytometry. Surprisingly, we did not see an increase in C3 deposition following incubation with CR2Fc but instead saw a slight decrease in C3 deposition (Figure 3; mean fluorescence 71.15 vs 93.72). This may be due to the fact that CR2Fc binds C3 and therefore may be covering the binding site for the C3-FITC antibody,

preventing detection of deposited C3 by this method. In addition, it may simply be caused by the need to optimize serum and antibody concentrations for the assay. We are currently attempting to resolve this problem. In order to further test the function of the recombinant protein *in vitro* we used a complement lysis assay. This was performed in two ways differing only in the method used to detect lysis**.** Both methods involved incubating the tumor cells with the complement activating antibody BCP8 and normal rat serum, in the presence or absence of CR2Fc. Detection of cell lysis was determined either by trypan blue staining or flow cytometry. Thus far we have not seen a significant increase in lysis *in vitro.* However, this could be due to the need for optimization of antibody and or serum concentrations for the assay as discussed above. Additionally, since CR2Fc is expected to increase antibody dependent cell cytotoxicity that would require additional effector cells we may not be able to detect this effect *in vitro* but would still expect to see a response *in vivo.* Our next step will be to determine whether CR2Fc enhances antibody dependent cell-mediated cytotoxicity *in vitro*. Finally we will determine the *in vivo* effect CR2Fc has on the immune response and its ability to increase tumor cell lysis.

**Key Research Accomplishments:**

- Construction, expression and purification of two CR2Fc construct (with or without linker)
- Optimization of CR2Fc purification for high yields and purity for use in animal studies
- Characterization of CR2Fc *in vitro*;
    - Showing targeting of CR2Fc to deposited C3 on tumor
    - Working out conditions and problems with C3 deposition and complement lysis assays

**Reportable Outcomes:**

- Rapisardo M, Ohta R and Tomlinson S. Enhancement of the immune response following immunization with a MUC1-C3d fusion protein. DOD Era of Hope BCRP meeting. Poster #P52-3. Baltimore, MD. June 25-28, 2008.

**Conclusions:**

In the second year of these studies the recombinant protein CR2Fc was successfully constructed, expressed and purified. The purification process has been optimized and stocks of both (linker and no linker constructs) recombinant proteins have been produced. The proteins have been characterized *in vitro* and shown to target (bind) to C3 deposited on tumor cells. Difficulties were encountered with C3 deposition and complement lysis assays but they are currently being optimized. Although further *in vitro* studies are planned, we can conclude from these studies that the recombinant protein is correct and functional. Next we will finish the *in vitro* characterization and proceed with *in vivo* studies to investigate the effects of CR2Fc on the immune response.

**Appendices:**


Figure 1. Western blot confirming identity of purified CR2Fc. Blot was stained with 7G6 (anti-CR2) antibody and shows a band at the expected molecular weight (54 Kd).

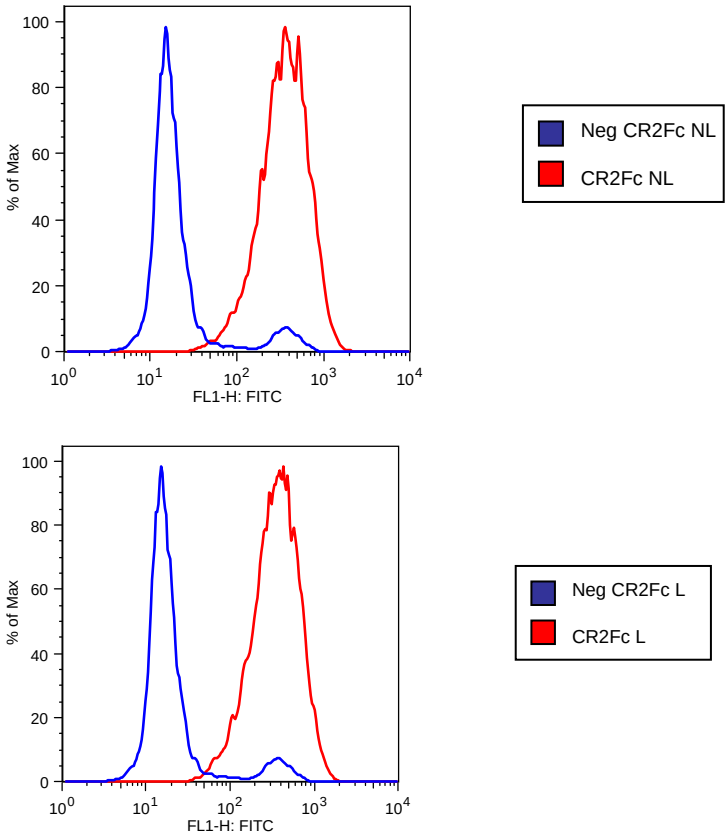

Figure 2. Binding of CR2Fc to CHO cells. The binding of both CR2Fc linker and no linker proteins to C3 opsonized CHO cells was shown by flow cytometry.

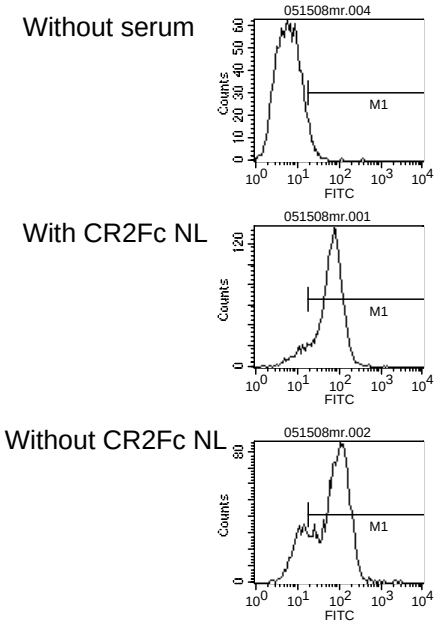


Figure 3. Complement deposition following incubation with CR2Fc. The ability of CR2Fc to increase C3 deposition on CHO cells was evaluated by flow cytometry. Mean fluorescence; with CR2Fc NL 71.15 vs without CR2Fc NL 93.72.